

Targeting COX-2 abrogates mammary tumorigenesis

Breaking cancer-associated suppression of immunosurveillance

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Three studies addressed the role of cyclooxygenase-2 (COX-2) in mammary tumorigenesis using epithelial and macrophage COX-2 knockout mice. Deletion of COX-2 in either cell restored, at least partially, tumor immunosurveillance either by changing macrophage function to offset pro-tumor effects, or by attracting more cytotoxic T lymphocytes and natural killer cells to the tumor. These studies suggest benefits from targeted COX-2 selective inhibition in combination with immunotherapies.

Epidemiological studies and clinical trials show therapeutic protection against cancers of the colon, prostate, lung, breast, and other tissues afforded by long-term use of cyclooxygenase-2 (COX-2) selective inhibitors, the ‘coxibs.’¹ These findings have been recapitulated across COX-2 deficient cancer mouse models, with suppressed COX-2-derived prostaglandin E₂, a dominant pro-inflammatory mediator that also promotes proliferation and angiogenesis while suppressing apoptosis and immune function, identified as the principal anticancer event.² However, emergence of significant cardiovascular risks, arising from collateral suppression of the cardioprotective endothelial COX-2 derived mediator prostacyclin, have raised doubts about the benefits of systemic COX-2 inhibition.³ We engineered mice lacking COX-2 selectively in distinct cellular compartments—mammary epithelium (COX-2 KO^{epi}) or myeloid cells (COX-2 KO^{myeloid})—to delineate the role of COX-2 in mammary tumorigenesis and to determine the protective effects of cell-specific COX-2 inhibition, thus avoiding systemic cardiovascular side-effects.^{4–6}

In COX-2 KO^{epi} mice mammary tumor onset, induced either by the carcinogen DMBA or transgenic expression of an activated HER2/neu oncogene, was delayed.^{4,5} Although qualitatively and quantitatively different, in both models significant changes were evident in the tumor microenvironment involving tumor-associated macrophages (TAM) and tumor-infiltrating lymphocytes (TIL), which are essential to immune destruction of tumor cells.

In the DMBA model, the dominant effect of epithelial COX-2 deletion was augmented TAM,⁴ a counterintuitive finding since TAM are typically of the M2 phenotype that promotes tumor growth by mechanisms that include suppression of innate and adaptive anti-tumor immunity.⁷ However, phenotypic marker and cytokine analysis revealed that COX-2 KO^{epi} mammary TAM were skewed toward the M1 phenotype that supports type 1 anti-tumorigenic CD4⁺ T helper type 1 lymphocytes (T_h1). Concordantly, increased CD4⁺ and correlation between M1-TAM and T_h1 markers, was detected in DMBA-induced

COX-2 KO^{epi} mammary tumors. In addition, natural killer (NK) cell markers were increased in COX-2 KO^{epi} tumors, further supporting a paracrine influence of epithelial cell COX-2 to suppress anti-tumor immune function.

In the HER2/neu model, which more closely resembles human disease, the phenotype was more profound – COX-2 KO^{epi} tumor onset was delayed, and multiplicity reduced, co-incident with decreased cell proliferation, suppressed angiogenesis, and a shift toward increased infiltrating CD3⁺CD4⁺ (likely T_h1), CD3⁺CD8⁺ (cytotoxic T-lymphocytes or CTL), and CD3⁺CD8⁺ (likely NK) immune cells.⁵ Mechanistically, this shift toward anti-tumor immune function appeared related not to TAM function but rather to modified COX-2 KO tumor cell function to produce more of the lymphocyte chemo-attractant CXCL-9 and less of the lymphocyte co-inhibitory molecule PD-L1. This novel link between COX-2 and PD-L1 function was confirmed when lower PD-L1 expression was observed in HER2/neu transformed mammary tumor cells transduced with shRNA to knock

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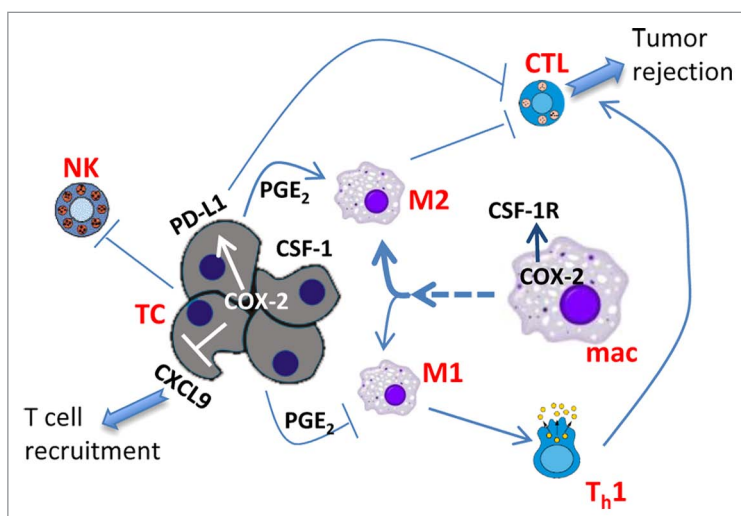


Figure 1. Cell-specific role of cyclooxygenase-2 (COX-2) in suppression of antitumor immune function. COX-2 in both tumor cells and macrophages may contribute to suppressed CTL function in mammary tumors. Tumor cell COX-2 may reduce expression of the T-cell attractant CXCL9, and enhance the lymphocyte co-inhibitory molecule PD-L1, thereby limiting lymphocyte infiltration and cytotoxic function. In addition, tumor cell COX-2 may skew macrophage polarization, limiting the antitumor pro-immune M1, and enhancing the pro-tumor immunosuppressive M2, phenotype. Macrophage COX-2 promotes CSF-1-dependent macrophage migration toward tumor cells as well as M2 polarization, thereby limiting the number and function of CTLs in tumors. TC = Tumor cell; mac = macrophage, M1 and M2 type; CTL = cytotoxic T cell; Th1 = helper T cell, type 1; NK = natural killer cell; CSF-1 = colony stimulating factor-1; CSF-1R = CSF-1 receptor; PGE₂ = Prostaglandin E₂; PD-L1 = programmed death-ligand 1.

down (KD) COX-2. Moreover, COX-2 KD cells failed to grow orthotopic tumors in wild type mouse fat pads. The critical role of CD8⁺ cells was apparent when CD8⁺ cell depletion in wild type mice restored COX-2 KD tumor growth to the level seen with normal tumor cells.

Together these studies indicated a significant role for tumor cell COX-2 in tumor immune suppression. Given the central role of TAM in the tumor immune microenvironment, and the central function of COX-2 in inflammatory macrophages, we developed HER2/neu transgenic mice lacking COX-2 in macrophages, as well as other myeloid cells (COX-2 KO^{myeloid}). Significant suppression of mammary tumor onset, multiplicity and growth was evident.⁶ Importantly, orthotopic tumor growth of normal mammary tumor cells was dramatically reduced in COX-2 KO^{myeloid} hosts compared with wild type, underscoring the significant pro-tumor role of myeloid/macrophage COX-2. Similar to the COX-2 KO^{epi} model, increased CD4⁺ and CD8⁺ TIL were evident in COX-2 KO^{myeloid} tumors. This appeared secondary to reduced

TAM-mediated T-cell suppression that was driven by impaired colony stimulating factor-1-dependent migration of COX-2 KO macrophages to tumors, as well as restrained differentiation to the immune-suppressive M2 TAM phenotype. Host depletion of CD8⁺, but not CD4⁺, cells restored orthotopic tumor growth in COX-2 KO^{myeloid} mice, again underscoring the contribution of COX-2 to suppressing the crucial anti-tumor role of CD8⁺ cells.

Immunosurveillance by CD8⁺ CTL and NK cells has emerged as critical for controlling nascent in situ tumor growth. This has led to a wave of promising new therapeutic strategies, including blockade of immune checkpoints such as CTLA-4, PD-L1, and its receptor PD-1, or adoptive transfer of anti-tumor T lymphocytes, with significant and durable clinical responses reported in trials.^{8,9} While the efficiency of COX-2 inhibition in promoting cytotoxic immune function should be verified in human breast and other cancers, our mouse studies implicate COX-2 in the 'broken' immune-surveillance that allows for mammary tumor progression (Fig. 1), and demonstrate that, rather

than the hazardous systemic inhibition, targeted cell-specific COX-2 inhibition is sufficient to achieve the benefit. These studies open possibilities for combination of COX-2 inhibitors to potentiate the clinical effect of checkpoint blockade or adoptive T-cell transfer. Remarkably, our studies show that targeted inhibition of COX-2 in macrophages, a component of the complex tumor microenvironment, produced substantial antitumor immune function and suppressed tumor growth, revealing opportunities to combine targeted delivery of COX-2 selective inhibitors directly to macrophages. One approach is to incorporate coxibs into HDL nanoparticles, which are readily engulfed by macrophages,¹⁰ to achieve the tumor suppressive effect of systemic COX-2 inhibition but without thromboembolic risk, because COX-2 function in healthy vascular endothelium is spared. With such targeted strategies, the promise of COX-2 inhibitors in cancer prevention and therapy, which was all but lost to the cardiovascular risk of systemic inhibition, may be renewed and realized.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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